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THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS ***US60/632,198***

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. §1.53(b)(2)

Atty. Docket: FISHMAN19

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[ ] Additional inventors are being named on separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

BIOLOGICAL MARKER FOR INFLAMMATION

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ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification — Number of Pages 18 — [X] Applicant claims small entity status. See 37 C.F.R. §1.27

[X] Drawing(s) — Number of Sheets 4 — [ ] Other (specify) _____

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[X] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 02-4035

The Invention was made by an agency of the United Stated Government or under a contract with an agency of the United States Government.

[X] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.

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BIOLOGICAL MARKER FOR INFLAMMATION

FIELD OF THE INVENTION

This invention relates to the fields of diagnosis and determining effectiveness of treatment and in particular to biological markers associated with inflammatory states.

PRIOR ART

The following is a list of prior art which is considered to be pertinent for describing the state of the art in the field of the invention. Acknowledgement of these references herein will at times be made by indicating their number within brackets from the list below.

1. Fishman P, Madi L, Bar-Yehuda S, Barer F, Del Valle L, Khalili K. Evidence for involvement of Wnt signaling pathway in IB-MECA mediated suppression of melanoma cells. *Oncogene*., 21:4060-4064 (2002).

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receptor fate and tumor growth inhibition. *J. Bio. Chem.*, 278:42121-42130 (2003).

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11. Madi L, Ochaion A, Rath-Wolfson L, Bar-Yehuda S, Erlanger A, Ohana G, Harish A, Merimski O, Barer F, Fishman P. The A3 Adenosine

Receptor is Highly Expressed in Tumor vs. Normal Cells: Potential Target for Tumor Growth Inhibition. *Clinical Cancer Research*, **10**: 4472-4479, 2004.

12. Gessi, S. et al. Elevated expression of A_3 adenosine receptors in human colorectal cancer is reflected in peripheral blood cells *Clinical Cancer Research* **10**:5895-5901, 2004

13. US Patent Application No. 20040137477 A1.

BACKGROUND OF THE INVENTION

The A_3 adenosine receptor, a G_i protein-associated cell surface receptor, has been found to be utilized as a target to combat cancer and inflammation. The receptor is highly expressed in various tumor cell types while low expression was shown in adjacent normal tissues. Activation of the receptor by a specific synthetic agonist induces modulation of downstream signal transduction pathways which include the Wnt and the NF-kB, resulting in tumor growth inhibition (1-5).

In vivo studies have shown that A_3AR agonists inhibit the development of colon, prostate and pancreatic carcinomas as well as melanoma and hepatoma.

A_3AR agonists were also been shown to act as anti-inflammatory agents by ameliorating the inflammatory process in different experimental autoimmune models such as rheumatoid arthritis and Crohn's disease (6-9). It was proposed also that the A_{2A} and A_3 receptors mediate the anti-inflammatory effects of methotrexate (10).

A_3 adenosine receptor (A3AR) expression levels are elevated in cancer cells as compared to normal cells (11,12). Thus, the A3AR expression level has been described as a means for the diagnosis of cancer (13). In addition, A3AR expression levels have also been described to be elevated in peripheral blood cells of patients with colorectal cancer (12).

SUMMARY OF THE INVENTION

It is an object of the invention to provide a method for determining an inflammatory state in a subject.

It is a further object of the invention to provide a method for determining the effectiveness of an anti-inflammatory therapeutic treatment of a subject.

The present invention is based on the surprising finding that there is an increase in the level of A_3 adenosine receptor expression in the WBC of a subject who has an inflammatory condition as compared to the WBC of a healthy subject. Furthermore, it was found that in subjects who respond to anti-inflammatory drug treatment, there is a reduction in the level of A_3 adenosine receptor expression in their WBC. This finding paves the way for the use of the A_3 adenosine receptor expression level as a means for the diagnosis of an inflammatory state, as well as other applications described below.

In a first aspect of the invention, there is provided a method of determining an inflammatory state in a subject, comprising:

(a) obtaining from the subject a sample comprising white blood cells (WBC); and

(b) determining the level of expression of A_3 adenosine receptor (A3AR) in the WBC of the sample, wherein a high level of expression is indicative of an inflammatory state in the subject.

In a second aspect of the invention, there is provided a method for determining the severity of an inflammatory state in a subject comprising:

(a) obtaining from the subject a sample comprising WBC;

(b) determining the level of expression of A3AR in the WBC of the sample;

(c) comparing the level of expression of A3AR in the cells with the level of prior determined or obtained standards, to determine the severity of the inflammatory state of the subject.

In a third aspect of the invention, there is provided a method for determining the effectiveness of an anti-inflammatory therapeutic treatment of a subject comprising:

(a) obtaining from the subject samples comprising white blood cells (WBC) from at least two discrete time points, at least one of which is during anti-inflammatory treatment; and

(b) determining the level of expression of A3AR in the WBC of the samples, wherein a difference is indicative of the effectiveness of the anti-inflammatory therapeutic treatment.

The term "*level of expression*" as used herein includes both the level of A3AR mRNA as well as the level of A3AR protein or A3AR protein fragments in the sampled cells.

The A3AR level of expression in WBC in accordance with some embodiments of the invention may be used for determination of the state or severity of inflammation, e.g. for determining the presence or absence of an inflammatory state. In accordance with other embodiments of the invention, the A3AR level of expression may be used for quantitative determination of the degree of severity of the inflammatory state. The term *"determining"* or *"determination"* will be employed below to refer to either or both quantitative or qualitative determination.

An "*inflammatory state*" includes any state of active or sub-clinical inflammation. The inflammation may be due to an inflammatory disease, or it may be a side effect of some other type of disease or disorder. Examples of inflammatory diseases include but are not limited to inflammatory bowel diseases, inflammatory corpuscle, inflammatory fibrous hyperplasia,

inflammatory gallbladder disease, inflammatory papillary hyperplasia and autoimmune diseases.

The autoimmune diseases may include any of the following: Myasthenia Gravis (MG), Congenital myasthenia gravis, Multiple sclerosis (MS), Stiff-man syndrome, Tropical spastic paraparesis, Rasmussen's encephalitis, Acute motor axonal neuropathy, Acute sensory-motor axonal neuropathy, Dorsal root ganglion neuritis , Acute pan-autonomic neuropathy, Brachial neuritis, Acute necrotizing hemorrhagic lekoencephalitis, Sporadic necrotizing myelopathy, Paraneoplastic cerebellar degeneration, Guillain-Barre syndrome, Limbic encephalitis, Opsoclonus-myoclonus ataxia, Sensory neuronitis, Autonomic neuropathy, Demyelinating neuropathy, AIDS-dementia complex, Tourette's syndrome, Miller-Fisher syndrome, Alzheimer's disease, Graves' Disease, Hashimoto's thyroiditis, Postpartum thyroiditis, Focal thyroiditis, Juvenile thyroiditis, Idiopathic hypothyroidism, Type I (insulin dependent) diabetes mellitus, Addison's disease, Hypophysitis, Autoimmune diabetes insipidus, Hypoparathyroidism, Pemphigus Vulgaris, Pemphigus Foliaceus, Bullous phemphigoid/ Pemphigoid gestationis, Cicatrical pemphigoid, Dermatitis herpetiformis, Epidermal bullosa acquisita, Erythema multiforme, Herpes gestatonis, Vitiligo, Chronic urticaria, Discoid lupus, Alopecia universalis/Areata, Psoriasis, Autoimmune hepatitis, Primary biliary cirrhosis, Chronic active hepatitis, Chronic active hepatitits/ Primary biliary cirrhosis overlap syndrome, Primary sclerosing cholangitis, Autoimmune hemolytic anemia, Idiopathic thrombocytopenic purpura, Evans syndrome, Heparin-induced thrombocytopenia, Primary autoimmune neutropenia, Autoimmune (primary) neutropenia of infancy, Autoimmune neutropenia following bone marrow transplant, Acquired autoimmune hemophilia, Autoimmune gastritis and pernicious anemia, Coeliac disease, Crohn's disease, Ulcerative colitis, Sialadenitis, Autoimmune premature ovarian failure, Azoospermia, Hypogonadism, Male infertility associated with

sperm autoantibodies, Autoimmune orchitis, Premature ovarian failure, Autoimmune oophoritis, Uveitis, Retinitis, Sympathetic ophthalmia, Birdshot retinochoroidopathy, Vogt-Koyanagi-Harada granulomatous uveitis, Retinal degeneration, Lens-induced uveitis, Optic neuritis, Autoimmune sensorineural hearing loss, Meniere's disease, Autoimmune myocarditis, Congenital heart block (neonatal lupus), Chagas' disease, Adriamycin cardiotoxicity, Dressler's myocarditis syndrome, Bronchial asthma, Interstitial fibrosing lung disease, Rapidly progressive glomerulonephritis, Autoimmune tubulointerstitial nephritis, Systemic lupus erythematosus (SLE), Antiphospholipid syndrome, Rheumatoid arthritis, Juvenile Rheumatoid arthritis, Felty's syndrome, Large granular lymphocytosis (LGL), Sjogren's syndrome, Systemic sclerosis (scleroderma), Crest syndrome, Mixed connective tissue disease, Polymyositis/dermatomyositis, Goodpasture's Disease, Wegener's granulomatosis, Churg-Strauss syndrome, Henoch-Schonlein purpura, Microscopic polyangiatis, Periarteritis nodosa, Bechet's syndrome, Atherosclerosis, Temporal (giant) cell arteritis, Takayasu arteritis, Kawasaki disease, Ankylosing spondilitis, Reiter's disease, Sneddons disease, Autoimmune polyendocrinopathy, candidiasis-ectodermal dystropy, Essential cryoglobulinemic vasculitis, Cutaneous leukocytoclastic angiitis, Lyme disease, Rheumatic fever and heart disease, Eosinophilic fasciitis, Paroxysmal cold hemoglobinuria, Polymyalgia rheumatica, Fibromyalgia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-spot and skin changes), Relapsing polychondritis, Autoimmune lymphoproliferative syndrome, TINU syndrome (acute tubulointerstitial nephritis and uveitis), Common variable immunodeficiency, TAP (transporter associated with antigen presentation) deficiency, Omenn syndrome, HyperIgM syndrome, BTK agammaglobulinemia, Human immunodeficiency virus and Post bone-marrow-transplant.

The sample comprising WBC used in the methods of the invention may include any of the known types of cells which make up this group. In particular, the sample should preferably include mononuclear cells (monocytes and/or lymphocytes). At times, the sample may include in addition, or in the alternative, granulocytes (neutrophils, eosinophils or basophils). The WBC may be obtained either from the blood of the subject, or from lymphatic tissue such as lymph nodes or spleen.

In the first aspect of the invention, a high level of expression of A3AR is employed as an indicator of an inflammatory state in the subject. The term "*high level*" is to be understood as meaning a significantly higher level of expression than in normal cells. For example, the level of the A3AR expression in the WBC may be compared to a control level, the control level being the level of A3AR expression in normal WBC of a healthy subject. At times it may be useful to determine the expression level by testing an assayed sample from an individual in parallel to one or more reference standards, e.g. one reference standard indicative of a normal sate and another indicative of an inflammatory state.

In the second aspect of the invention, the determined expression level is compared to standards. The standards may be based on previously determined levels from healthy individuals and from individuals with an inflammatory state or with different inflammatory states. The standards may be provided, for example, in the form of discrete numeric values or, in case the assay method is colorimetric, in the form of a chart with different colors or shadings for healthy and inflammatory states; or they may be provided in the form of a comparative curve prepared on the basis of such standards.

Such standards may be prepared by determining the level of A3AR expression (which may be the level of A3AR protein, protein fragment, or mRNA level etc., as discussed above) present in WBC cells obtained from a plurality of patients positively diagnosed (by other means, for example by a

physician, by histological techniques etc.) as having inflammation at varying levels of severity. The severity of the disease for the preparation of the standards may also be determined by various conventional methods such as by pathological techniques. In another embodiment, the assay is carried out in parallel to a number of standards of healthy subjects and subjects of different inflammatory states and the level determined in the assayed sample is then compared to such standards.

For example, a protein content level of between X_1 to X_2 per 1,000,000 cells may be defined as being indicative of grade 1 inflammation, a higher protein content of Y_1 to Y_2 per 1,000,000 cells may be defined as being indicative of grade 2 inflammation, etc. After such a standards are prepared, it is possible to compare the level of A3AR expression obtained from a specific individual to the corresponding value of the standards, and thus obtain an assessment of the severity of the disease.

In the third aspect of the invention, the effectiveness of an anti-inflammatory therapeutic treatment of a subject may be assessed by taking samples of WBC at various time points before, during and after the treatment. For example, a first sample may be taken at a time point prior to initiation of the treatment and a second sample may be taken at a time point during the treatment. A decrease in the level of the A3AR expression in the second sample as compared to the first sample would be indicative that the treatment is effective. The degree of decrease could be indicative of the degree of effectiveness of the treatment, i.e. the correlation would be quantitative.

In another example, a first sample may be taken at a time point during the treatment and a second sample may be taken at a time point during the treatment subsequent to the time point of the first sample. A decrease in the level of the A3AR expression in the second sample as compared to the first sample would be indicative that the treatment is effective.

In a third example, a first sample may be taken at a time point during the treatment and a second sample may be taken at a time point after the treatment has been discontinued. In this case, an increase in the level of the A3AR expression in the second sample as compared to the first sample would be indicative that the treatment is effective.

Of course, various other combinations may be carried out, as well as the taking of samples at more than two time points.

The therapeutic treatment may be any type of treatment given to a patient with an inflammatory condition, including drug treatment. Drugs for treatment of inflammatory conditions are well know and include disease modifying drugs such as Methotrexate or prednisone; non steroidal anti-inflammatory drugs (NSAIDs) such as Diclofenac, Diflunisal, Etodolac, Fenoprofen, Flurbiprofen, Ibuprofen, Indomethacin, Ketoprofen, Meclofenamate, Nabumetone, Naproxen, Oxaprozin, Phenylbutazone, Piroxicam, Sulindac, Tenoxicam, Tiaprofenic Acid, or Tolmetin; anti-TNF drugs such as etanercept, adalimumab, or infliximab; and A3AR agonists such as N6-(3-iodobenzyl) adenosine-5'-N-methyl- uronamide (IB-MECA) or 2-chloro-N^6-(3-iodobenzyl)-adenosine-5'-N-methly-uronamide (Cl-IB-MECA); as well as others.

BRIEF DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is a graph showing the change in severity of arthritis as a function of time in control animals and in animals treated with MTX or IB-MECA;

Fig. 2 is a bar graph and Western blot showing A3AR protein expression level in paw (A) and synovial tissue (B) under various conditions;

Fig. 3 is a bar graph and Western blot showing A3AR protein expression level in lymph node (A) and spleen (B) cells under various conditions;

Fig. 4 is a bar graph and Western blot showing A3AR protein expression level in lymph nodes under various conditions;

Fig. 5 is a bar graph and Western blot showing A3AR protein expression level in healthy subjects and in RA patients; and

Fig. 6 is a table illustrating the response of RA patients to treatment with IB-MECA.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Materials and Methods

Induction of adjuvant induced arthritis (AIA) model in rats

Female Lewis rats, aged 8-12 weeks were obtained from Harlan Laboratories (Jerusalem, Israel). Rats were maintained on a standardized pelleted diet and supplied with tap water. Experiments were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee at Can-Fite BioPharma, Petah Tikva, Israel. The rats were injected subcutaneously (SC) at the tail base with 100 µl of suspension composed of incomplete Freund's adjuvant (IFA) with 10 mg/ml heat killed Mycobacterium tuberculosis, (Mt) H37Ra, (Difco, Detroit, USA). Each group contained 10 animals.

Treatment with IB-MECA (10 µg/kg) was initiated on day 14 after vaccination and was orally administered by gavage, twice daily. Another group was treated with Methotrexate (MTX) (1.5 mg/kg) intraperitoneally every 3 days, starting on day 14th after vaccination. The control group in each

experiment received vehicle only (DMSO in a dilution corresponding to that of the drugs).

Clinical Disease Activity Score was assessed as follows: the animals were inspected every second day for clinical arthritis. The scoring system ranged from 0-4 of each limb: 0- no arthritis; 1- redness or swelling of one toe/finger joint; 2- redness and swelling of more than one toe/finger joints, 3- the ankle and tarsal-metatarsal joints involvement. 4- entire paw redness or swelling. The clinical score was calculated by adding the four individual legs' score. The inflammatory intensity was also determined in accordance with the increase in the rat hind paw's diameter, measured by caliper (Mitotoyo, Tokyo, Japan).

Separation of inflammatory and hematopoietic tissues and preparation of protein extracts

a. Inflammatory Tissues

The hind paws were dissected above the ankle joint. The bony tissue was broken into pieces, snap frozen in liquid nitrogen and stored at -80°C until use. To prepare a protein extract, RIPA buffer (containing 150mM NaCl, 50mM Tris, 1% NP40, 0.5% Deoxycholate and 0.1% SDS) was added to the paw tissue (4 ml/gr of tissue). The mixture was homogenized on ice with a polytron and centrifuged.

Synovial tissue was removed and synovial cells were separated by incubating the tissue in RPMI containing 1 mg/ml Collagenase IV and 0.1mg/ml DNase with a vigorous shaking (200 rpm) at 37°C for 30 min. The supernatant containing the synovial cells was collected and the undigested tissue was re-extracted. The supernatants from both extractions were combined and cells were washed with PBS. Protein extracts were prepared.

b. Hemopoietic Tissues

Lymph nodes were removed and cells were separated by first mincing the tissue and disaggregating it through a needle of 22 G. Spleens were removed and subjected to Lymphoprep (Nycomed AS, Oslo, Norway) for mononuclear cell separation. Protein extracts were prepared.

Separation of peripheral blood mononuclear cells from RA patients and healthy subjects

Blood was withdrawn from healthy subjects or RA patients. Mononuclear cells (lymphocytes and monocytes) were separated using Ficoll-Hypaque gradient. Protein was extracted from the mononuclear cells.

Clinical Study

Blood was withdrawn from RA patients who were enrolled in a clinical study in which the effect of IB-MECA on arthritic patients was evaluated. The patients randomly received 0.1, 1.0 or 4.0 mg twice daily of IB-MECA. Blood was withdrawn at 2 time points: a) after 1 month of washout period from a previous treatment and before IB-MECA treatment was initiated – this was considered as base line level. b) after 3 months of treatment with IB-MECA. Peripheral blood mononuclear cells were separated and protein was extracted as described above. In addition, C reactive protein (CRP) values were analyzed and ACR was calculated for each patient (ACR is a parameter which was determined by the American College of Rheumatology to predict the response of patient to a given drug; ACR 20, ACR 50 and ACR 70 respectively represent a 20%, 50% and 70% improvement in these parameters).

Analysis of A3AR protein expression level by Western Blot (WB) analysis

Western blot analysis (WB) of synovial, paw, spleen and lymph nodes were carried out according to the following protocol. Samples were rinsed with ice-cold PBS and transferred to ice-cold lysis buffer (TNN buffer, 50mM Tris buffer pH=7.5, 150mM NaCl, NP 40). Cell debris was removed by centrifugation for 10 min, at 7500xg. Protein concentrations were determined using the Bio-Rad protein assay dye reagent. Equal amounts of the sample (50μg) were separated by SDS-PAGE, using 12% polyacrylamide gels. The resolved proteins were then electro-blotted onto nitrocellulose membranes (Schleicher & Schuell, Keene, NH, USA). Membranes were blocked with 1% BSA and incubated with the primary antibody against A3AR (dilution 1:1000) for 24h at 4°C. Blots were then washed and incubated with a secondary antibody for 1h at room temperature. Bands were recorded using BCIP/NBT color development kit (Promega, Madison, WI, USA).

RESULTS

IB-MECA inhibits the development of AIA

About 21 days after immunization, most of the vehicle treated animals progressively developed arthritis. IB-MECA treatment (10 μg/kg, given orally twice daily, starting on day 14th after immunization) and MTX treatment resulted in a significant decrease in disease severity as was evaluated by the arthritis clinical score. Disease peaked on days 21-28 and maximal effect of IB-MECA or MTX was seen on these days (Figure 1).

A_3AR is highly expressed in inflammatory tissues and in peripheral hematopoietic tissues of AIA rats

Low A3AR expression level was detected in the healthy paw & synovial tissues. In the inflammatory tissues derived from AIA rats, a marked increase in the A3AR protein expression level was noted. However, upon IB-MECA treatment A3AR level was down-regulated (Figures 2A & B). Interestingly, a similar pattern was noted in the peripheral hematopoietic tissues, i.e., low A3AR expression level was noted in the spleen and lymph node (LN) derived from naïve animals, high in the tissues from AIA and low expression in the tissues of IB-MECA treated rats (Figures 3A and B). In LN derived from AIA rats treated with MTX, a similar A3AR expression profile was observed (Figure 4).

High A3AR expression is found in MNC derived from RA vs. low in healthy subjects

Low A3AR expression level was found in MNC from healthy subjects whereas high expression was detected in MNC derived from RA patients (Figure 5).

In 4 patients that were treated with IB-MECA for a period of 3 months, down-regulation of A3AR expression was noted in comparison to A3AR expression level at baseline. In all 4 patients a decrease in CRP level was noted and an improvement in either ACR 20, 50 and 70 was seen (Figure 6). The direct correlation between the clinical response and receptor down-regulation led to the conclusion that A3AR expression may be suggested as a surrogate marker to predict response to a given anti-inflammatory treatment. A support for this notion was obtained from the data of patient number 5 which showed low A3AR expression at the baseline time point and did not respond clinically to IB-MECA treatment.

CLAIMS:

1. A method of determining an inflammatory state in a subject, comprising:

(a) obtaining from the subject a sample comprising white blood cells (WBC); and

(b) determining the level of expression of A_3 adenosine receptor (A3AR) in the WBC of said sample, wherein a high level of expression is indicative of an inflammatory state in the subject.

2. The method of claim 1 wherein the level of said A3AR expression in the WBC is compared to a control level, the control level being the level of A3AR expression in normal WBC of a healthy subject, or being a standard reference level for the A3AR expression which is indicative of a normal state.

3. The method according to claim 1, wherein the inflammatory state is the result of an autoimmune disease.

4. The method according to claim 3, wherein the autoimmune disease is rheumatoid arthritis (RA).

5. A method for determining the severity of an inflammatory state in a subject comprising:

(a) obtaining from the subject a sample comprising WBC;

(b) determining the level of expression of A3AR in the WBC of said sample;

(c) providing a calibration curve of the level of A3AR in said cells correlated to the severity of the inflammatory state; and

(d) comparing the level of expression of A3AR in said cells with the levels appearing in the calibration curve, thereby determining the severity of the inflammatory state of the subject.

6. The method according to claim 5, wherein the inflammatory state is the result of an autoimmune disease.

7. The method according to claim 6, wherein the autoimmune disease is rheumatoid arthritis (RA).

8. A method for determining the effectiveness of an anti-inflammatory therapeutic treatment of a subject comprising:

(a) obtaining from the subject samples comprising white blood cells (WBC) from at least two discrete time points, at least one of which is during the anti-inflammatory treatment; and

(b) determining the level of expression of A3AR in the WBC of the samples, wherein a difference is indicative of the effectiveness of the anti-inflammatory therapeutic treatment.

9. The method of claim 8 wherein a first sample is taken at a time point prior to initiation of the treatment and a second sample is taken at a time point during the treatment, and wherein a decrease in the level of the A3AR expression in the second sample as compared to the first sample is indicative that the treatment is effective.

10. The method of claim 8 wherein a first sample is taken at a time point during the treatment and a second sample is taken at a time point during the treatment subsequent to the time point of the first sample, and wherein a decrease in the level of the A3AR expression in the second sample as compared to the first sample is indicative that the treatment is effective.

11. The method of claim 8 wherein a first sample is taken at a time point during the treatment and a second sample is taken at a time point after the treatment has been discontinued, and wherein an increase in the level of the A3AR expression in the second sample as compared to the first sample is indicative that the treatment is effective.

12. The method according to claim 8 wherein said therapeutic treatment involves an anti-inflammatory drug.

13. The method according to claim 8, wherein the inflammatory state is the result of an autoimmune disease.

14. The method according to claim 13, wherein the autoimmune disease is rheumatoid arthritis (RA).

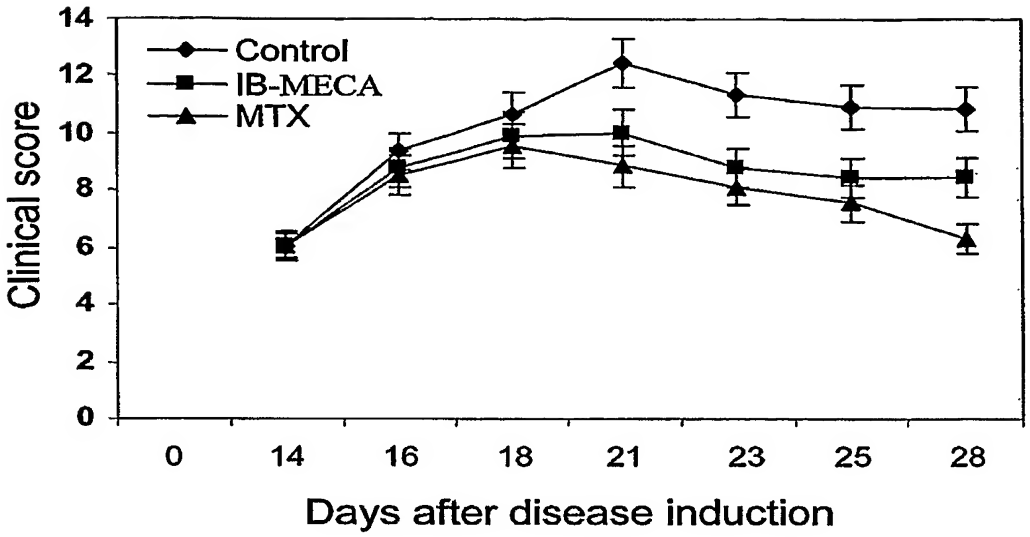


FIG. 1

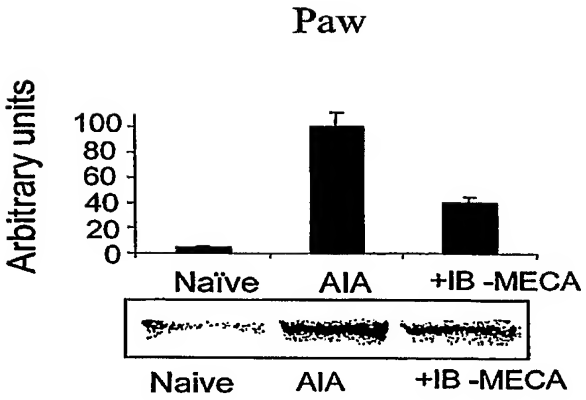


FIG. 2A

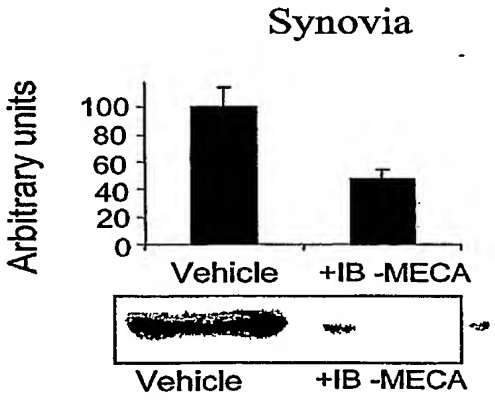


FIG. 2B

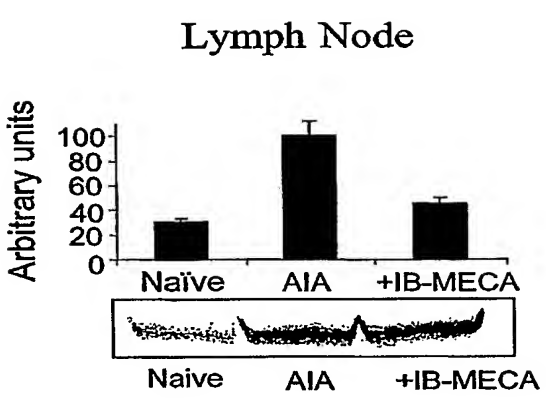


FIG. 3A

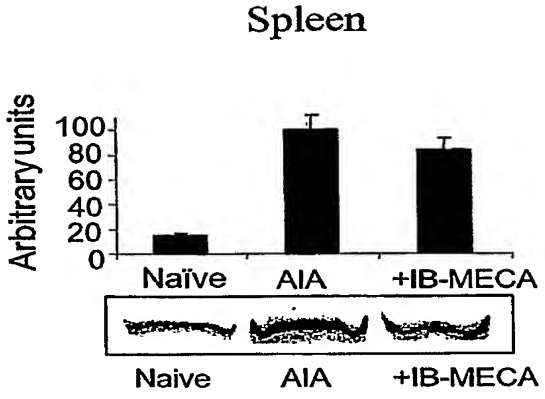


FIG. 3B

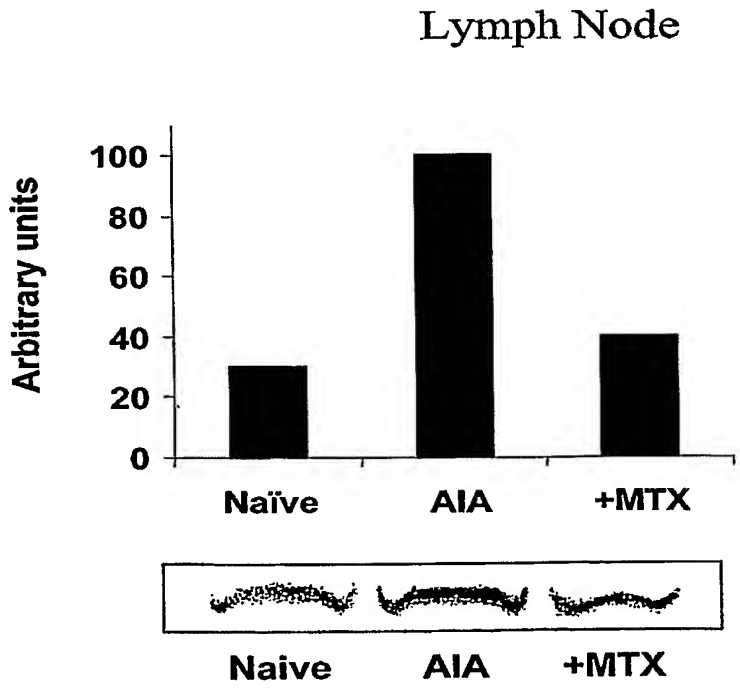


FIG. 4

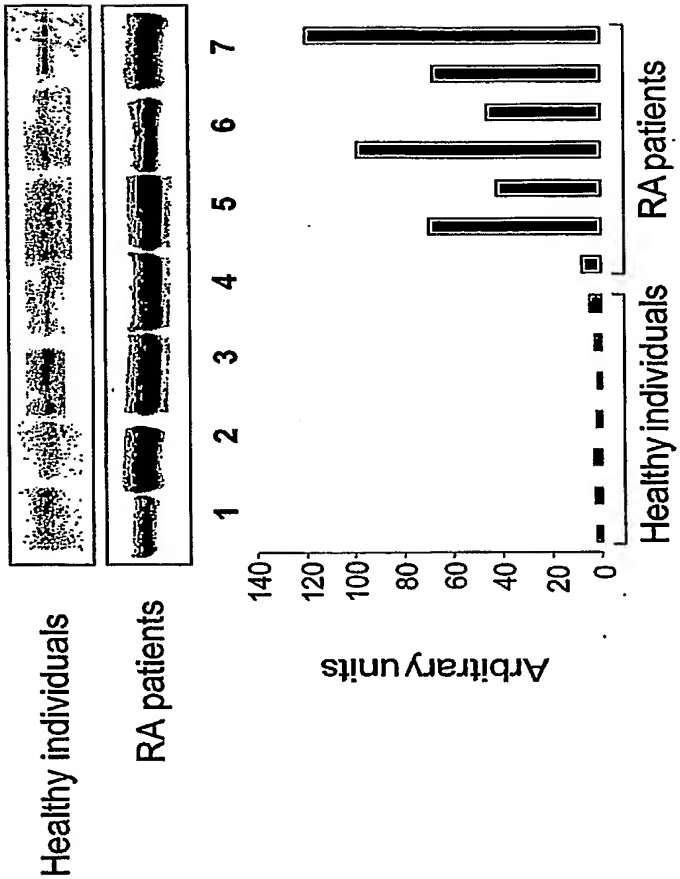


FIG.5

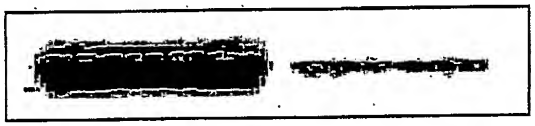
RA -patients	Baseline	IB - MECA 3 months Treatment	ACR 3 months Treatment	CRP Improvement 3 months (% of change)
Patient 1			70	43%
Patient 2			50	43%
Patient 3			20	90%
Patient 4			70	13%
Patient 5			-	-85%

FIG.6